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Research Article

The Effect of Storage Environment on Seed Survival and The Accumulation of Chromosomal Aberrations in Pea Landraces and Cultivars (*Pisum sativum* L.)

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Abstract: The relationship between loss of seed viability and the accumulation of chromosomal aberrations was investigated in both landraces and cultivars of the pea (*Pisum sativum* L.). It was shown here, for the first time with pea landraces, that loss of seed viability is associated with an increase in the frequency of chromosomal abberrations, and that even small losses of viability result in some chromosomal damage in the seeds of pea landraces as well as in the seeds of pea cultivars. In addition, for a given loss of viability the frequency of chromosomal aberrations in either low or high moisture content seed lots was the same. However, it was shown that under identical storage conditions, low moisture content seeds of the laudraces exhibit greater longevity than those of the cultivars. Following ageing, chromatid-type aberrations (in particular single fragments) were most frequently observed in the surviving seeds of both pea cultivars and landraces. The relevance of these findings to the long term conservation of pea germplasm is discussed.

Key Words: *Pisum sativum,* pea, seed viability, chromosomal aberrations.

Bezelye (*Pisum sativum* L.) Yerel Populasyonlarında ve Kültür Çeşitlerinde Depolama Ortamının Tohum Canlılığı ve Kromozomal Bozulmaların Birikimi Üzerine Etkisi

Özet: Bezelye (*Pisum sativum* L.) yerel populasyonlarında ve kültür çeşitlerinde tohum canlılığının kaybı ve kromozomal bozulmaların birikimi arasındaki ilişki araştırılmıştır. Burada, bezelye yerel populasyonlarında ilk defa gösterilmektedir ki; tohum canlılığının kaybı, kromozomal bozulmaların miktarındaki artış ile ilişkili olmaktadır ve hatta canlılıktaki ufak kayıplar bile bezelye çeşitlerinin tohumlarında olduğu gibi bezelye yerel populasyonlarının tohumlarında da kromozomal zararlanmaya neden olmaktadır. Buna ilave olarak, belirli bir canlılık seviyesi için düşük ya da yüksek nem kapsamındaki tohum gruplarında kromozomal bozulmaların miktarı aynı olmuştur. Fakat, bezelye yerel populasyonlarının düşük nem kapsamındaki tohumlarının, benzer depolama koşullarında, kültürü yapılan bezelye çeşitlerinin tohumlarından daha yüksek canlılıkta oldukları gösterilmiştir. Kültürü yapılan bezelye çeşitleri ve bezelye yerel populasyonlarının canlı tohumlarında, yaşlanmayı takiben, en çok kromatid-tipi bozulmalar (özellikle tek parça halindeki bozulmalar) gözlenmiştir. Bezelye genetik materyalinin uzun süreli saklanması ile ilgili olarak bu bulguların uygunluğu tartışılmıştır..

Anahtar Sözcükler: *Pisum sativum,* bezelye, tohum canlılığı, kromozomal bozulmalar.

Introduction

Many previous studies (1-3) have shown that, in a range of species, there is a close relationship between loss of seed viability during storage and the accumulation of genetic damage in the surviving seeds. The main factors affecting this relationship are seed moisture content, temperature and storage period, i.e. increasing either results in an increase in loss of seed viability or an increase in the frequency of chromosomal aberrations (4). However, so far, this relationship has only been examined in the seeds of cultivars. This study was the first attempt to investigate the effect of storage conditions on loss of viability and the accumulation of chromosomal aberrations in pea landraces in comparison with pea cultivars to determine if the same relationship holds for seeds of landraces.

Previously, D'Amato (5) and Abdalla and Roberts (6) have reported mainly chromosome-type aberrations in the root-tips of aged pea seeds. However, recently, Dourado and Roberts (7) have concluded that the majority of aberrations induced by ageing were of the chromatid-type, and the proportion of these to chromosome-type aberrations remained more or less constant with loss of viability. Therefore, in this research chromosomal damage induced both in seeds of pea landraces and cultivars under various storage conditions was examined to determine the type, frequency and changes in the frequency of chromosomal damage with severity of ageing.

Recent work on the lettuce (3) has shown that for a given loss of viability more chromosome-type aberrations are induced at low moisture contents (5.5%) than at high moisture contents (13.0%). This study was also conducted to determine if this same relationship applies to pea cultivars and landraces.

Materials and Methods

The pea seeds in this study represented two categories: cultivars and landraces. These two categories were chosen in order to compare their characteristics in terms of genetic deterioration during storage. Seeds of the cultivar "Kelvedon Wonder" had an initial moisture content of 11.5% and 95.5% normal germination (morphologically normal seedlings). Seeds of the cultivar "Douce Provence" had an initial moisture content of 15.1% and exhibited 96.5% normal germination.

A very limited amount of germplasm of pea landraces, JI 181 and JI 1104 (originating from Nepal), were obtained from the John Innes Institute, Norwich, England. Only limited information is avaliable on these landraces. JI 181 and JI 1104 are examples of Keerau peas and would be considered closer to the ecotypes of peas of this region with a rather small geographic distribution high plateaus in the Himalayas). The seed lots were regenerated (bulked up) twice in order to produce sufficient seeds for experimentation. The initial moisture contents of JI 181 and JI 1104 were 10.8% and 10.1%, respectively. All seed lots of the landraces had 100.0% normal germination.

Until required for experimentation, all the seed lots were stored in hermetically sealed laminated aluminum foil packets either in a cold room or in a fridge, both at 3°C.

To obtain low moisture content seed lots, the seeds were dried at 20°C over regularly regenerated silica gel in a partial vacuum in desiccators for approximately 6 and 10 months, for cultivars and landraces, respectively. The seed moisture contents of the pea cultivars and landraces were determined by the High Constant Temperature Oven Method, i.e. the seeds were dried for 1 hour at 130-133°C (8, 9). Moisture contents were expressed as percentages of fresh weight and represented means of two determinations.

High and low moisture content seed lots of the cultivars and landraces were aged under different storage conditions (for different periods at various combinations of moisture and temperature) to provide a range of viabilities in each seed lot using the improved viability equation (10, 11) and these assumed viability constants for pea seeds: K_E=9.868, C_W=5.389, C_H=0.0328 and C_Q=0.000481.

After storage, non-aged and aged seed lots of the pea cultivars and landraces were set to germinate between moist, rolled paper towels at 20°C according to the International Seed Testing Association (ISTA) Rules (8, 9) with the modification of eight 25-seed replicates and of an increase in the test duration from 8 days to 11 days in aged cultivars and 14 days in aged landraces, in the case of the high moisture content seed lots, and 14 days in aged cultivars and 21 days in aged landraces, in the case of the low moisture content seed lots. Any hard seeds remaining in the tests after 8 and 11 days were counted and then scarified with sandpaper, and the germination tests extended by a further 6 and 10 days in the seed lots of cultivars and landraces, respectively.

Chromosomal aberrations induced during storage of pea cultivars and landraces were estimated by examining the first mitotic divisions in the radicle tips of the germinating seeds. A number of sampling times and radicles of varying lengths were used depending on the percentage viability of each seed lot. In order to examine the maximum number of cells at first mitosis where normal germination was 80% or more, radicles of 4-8 mm in length were collected. Since the occurrence of the first mitotic division is delayed in relation to radicle extention in aged seeds (12), radicles of 9-13 mm in length were used when viability was between 50 and 80%. With the more severely aged pea treatments (i.e. seeds aged to below 50% viability), radicles were sampled at 14-18 mm in light of the observations by D'Amato (5) and Dourado and Roberts (7).

The excised radicles were placed in individual glass vials and fixed with glacial acetic acid: absolute alcohol mixture at a ratio of 1:3 for 15 minutes at room temperature. They were then rinsed in distilled water twice and stored in a solution of 70% ethanol at 3°C.

For slide preparation, the radicles were hydrolysed with 1M HCl in a water bath at 60°C for 10 minutes. The acid was then drained off and Schiff's Reagent (Feulgen), added for staining. The radicles were left to stain for 20 minutes in darkness at room temperature. A single radicle was transferred to a glass slide and a 0.8-1.0 mm portion excised from the radicle-tip for examination. One drop of 60% lacto-propionic-orcein was added and radicle-tip squashes were made according to the method described by Dyer (13).

Observations were restricted to chromosomal aberrations at late anaphase, since generally, chromosomal aberrations are more easily detected at this stage and also because of the relative ease of the procedure when examining large quantities of material. All late anaphases present in each slide were scored; a minimum of 400 anaphases per treatment were examined. For each treatment between 5 and 12 slides were examined.

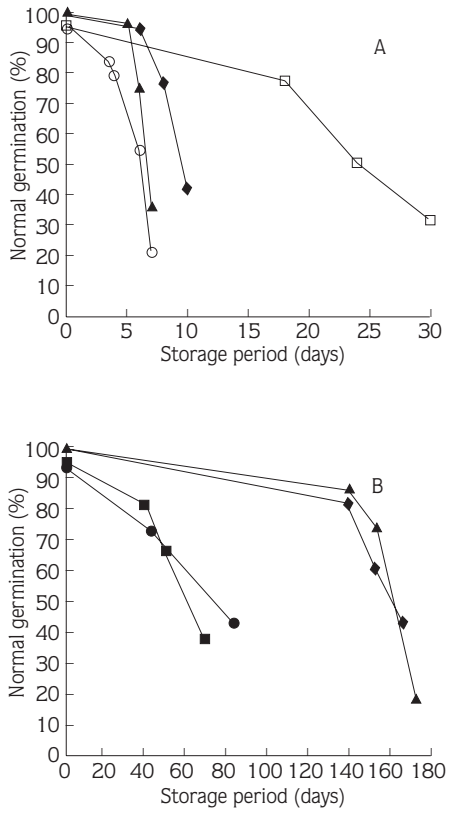

Figure 1. The relationship between storage period and percentage normal germination in seeds of pea cultivars and landraces stored under the following conditions:

A (high moisture contents)	B (low moisture contents)
(❑) Kel. Won., 50°C/11.5%	(■) Kel. Won., 65°C/4.7%
(❍) Dou. Pro., 50°C/15.1%	(●) Dou. Pro., 65°C/4.8%
(▲) JI 181 , 65°C/10.8%	(▲) JI 181 , 65°C/5.1%
(◆) JI 1104 , 65°C/10.1%	(◆) JI 1104 , 65°C/5.1%

The chromosomal aberrations observed were classified into one of the four categories: chromosome or chromatid-types, mixed or others.

Chromatid-type aberrations comprised of: single fragments, two or more fragments of unequal size; single bridges, two or more bridges with or without fragments of unequal size. *Chromosome-type* aberrations comprised of one or more double fragments; one or more double bridges with or without double fragments. Where both chromatid and chromosome-type aberrations were observed within a cell, the damage was classified as *mixed.* A small category, *other* damage including lagging chromosomes with or without any of the other groups were also recorded.

The percentage of aberrations in each category was calculated for various viability percentages along the survival curve.

In order to linearize the relationship between normal germination and storage period (Figures 2 and 3) and between aberrant anaphase cells and normal germination (Figure 5), percentages were transformed to probit values using a probit link function in the Genstat 5 computer program. Then, the above relationships were analysed with linear regression techniques. Initially, regression analysis was carried out separately for each of the eight storage conditions. Where the individual regression lines fitted to the relationship showed similar intercepts and slopes, a single line was fitted to represent the relationship within cultivars or landraces or the overall relationship. The best fitted lines to the relationships shown in Figure 3 (one each for cultivars and landraces) and Figure 5 (a single line for both cultivars and landraces) were also computed using the probit link function in the aforementioned computer program.

Results

i. The effect of storage on the viability of high and low moisture content seeds

Pea seeds (i.e. two landraces and two cultivars) were aged by storing hermetically for different periods at various temperatures and moisture contents. The ageing treatments decreased the viability of all seed lots of the pea landraces and cultivars with increases in time. The fastest decrease in germination was achieved by storing the seeds at high moisture contents and high temperatures. The percentages of normal germination after storage were plotted against time for each cultivar and landrace. This same relationship was true for the

cultivars Kelvedon Wonder and Douce Provence (Figure 1 A and B).

Both high and low moisture content seed lots of pea landraces, JI 181 (10.8% and 5.1%, respectively) and JI 1104 (10.1% and 5.1%, respectively) were aged at 65°C. The viability curves of the pea landraces (Figure 1A and B), showed that loss of seed viability was a function of storage environment, i.e. increasing seed moisture content, storage temperature and duration caused a decrease in seed viability. As expected, seed deterioration was fastest in the high moisture content seed lots, e.g. in JI 181, 10.8% moisture content, it took 7 days for viability to fall to 36% whilst at 5.1% moisture content it took 170 days, i.e. almost a twenty-four fold increase in time.

In addition, the percentage of normal germination of each seed lot was plotted on a probit scale against storage period. Figures 2 and 3 show that there was a linear relationship between the probit of percentage of normal germination and storage period under all the storage conditions.

Figure 2 shows that either high moisture contents or high temperatures resulted steep slopes for the seed lots. Thus, illustrating that survival was shortest under the harsher conditions, whilst seeds aged at 50°C and 11.5% moisture content took the longest to die.

Furthermore, Figure 3 is convincing evidence that in low moisture content pea seeds, loss of probit viability in landraces follows a similar trend to that of cultivars under similar storage conditions (i.e. 65°C and ca.5% seed moisture content) since there is no significant difference between the slopes of the cultivars and landraces ($P>0.05$). The slope of the best fitted regression line for the cultivars (-0.023) is almost parallel to that of the landraces (-0.025). However, the intercept of the line for pea landraces (4.096) is higher than that of the cultivated peas (1.659). This suggests that the storage life of low moisture content pea landraces is longer than that of pea cultivars when stored under similar conditions. The landrace peas deteriorate in the same manner as cultivars but at a slower rate.

ii. The effect of temperature, moisture content and time on the relationship between loss of seed viability and the accumulation of chromosomal aberrations

The frequency of visible chromosome damage (as percentage of aberrant anaphase cells at first mitosis) in the radicle tips of the surviving seeds were plotted against storage period (Figure 4). It is clear that in both landraces and cultivars, the frequency of aberrant cells increased with increases in storage period. Figure 4A shows that at high seed moisture contents the rate of accumulation of chromosomal aberrations in pea cultivars stored at 50°C and also in pea landraces stored at 65°C increased. For example, in Douce Provence, the frequency of aberrations increased from 3% to 25% and in Kelvedon Wonder from 4% to 21%. Similarly in JI 181, chromosomal aberrations increased from 0.9% to 19% and in JI 1104 from 0.6% to 17%. However, in the low moisture content seed lots (Figure 4B), pea cultivars showed a faster increase in the accumulation of aberrant anaphase cells compared with the pea landraces although they were stored at the same temperature, i.e. 65°C, and similar

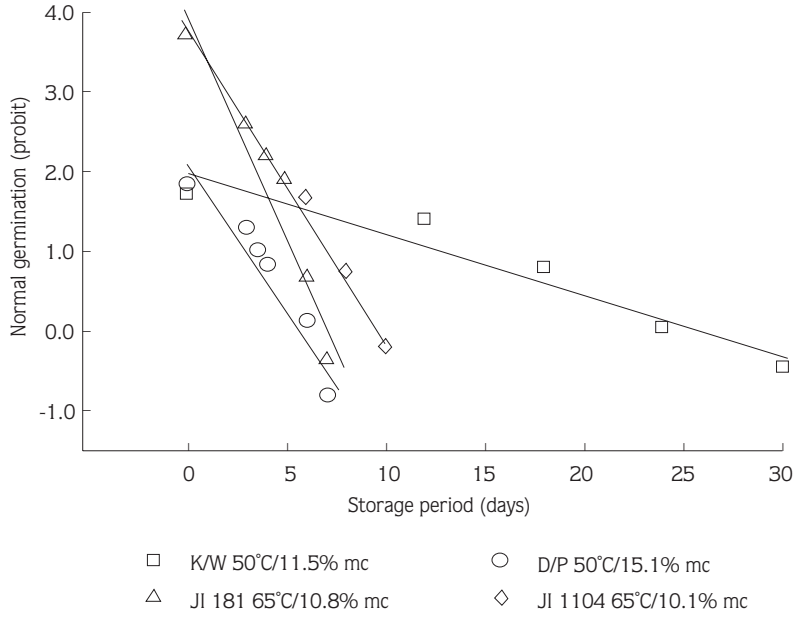

Figure 2. The relationship between storage period and normal germination (probit values) in the high moisture content seeds of pea cultivars and landraces stored at two different temperature regimes.

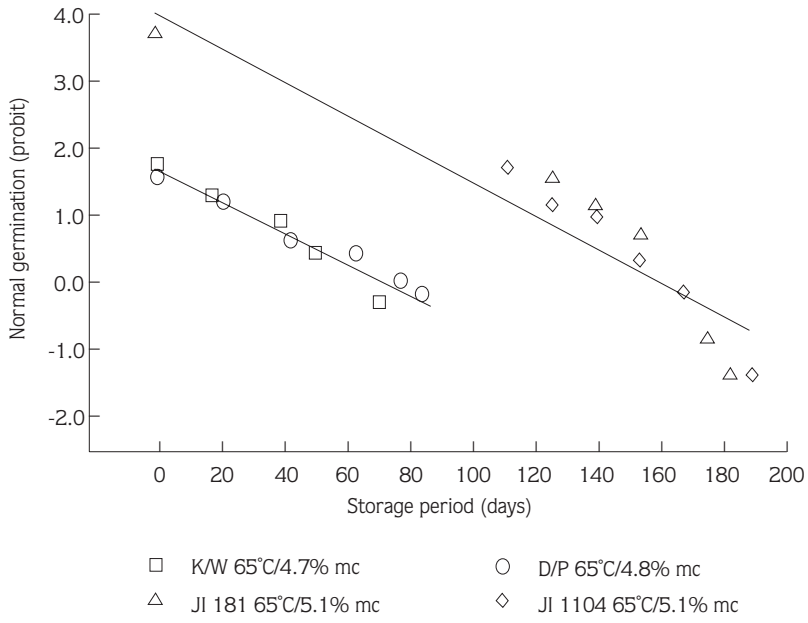

Figure 3. The relationship between storage period and normal germination (probit values) in the low moisture content seeds of pea cultivars and landraces stored at 65°C.

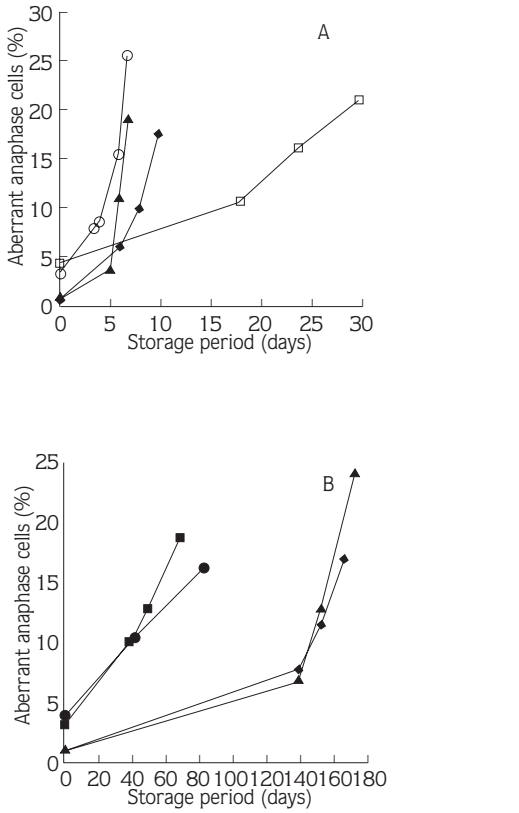

Figure 4. The relationship between storage period and the frequency of aberrant anaphase cells in surviving seeds of pea cultivars and landraces stored under the following conditions:

A (high moisture contents)	B (low moisture contents)
(❑) Kel. Won., 50°C/11.5%	(■) Kel. Won., 65°C/4.7%
(○) Dou. Pro., 50°C/15.1%	(●) Dou. Pro., 65°C/4.8%
(▲) JI 181 , 65°C/10.8%	(▲) JI 181 , 65°C/5.1%
(◆) JI 1104 , 65°C/10.1%	(◆) JI 1104 , 65°C/5.1%

moisture contents (about 5% moisture content) due to the differences in longevity already described. However, for a given loss of viability a similar frequency of aberrations was noted, i.e. for viability about 45%, Douce Provence had 16% aberrations whilst JI 1104 had 17%.

The percentage of normal germination and the total frequency of aberrant anaphase cells in each seed lot (irrespective of type) from the various storage conditions were plotted on probit scales in order to define the relationship between seed viability and the frequency of aberrant anaphase cells under the various storage conditions (Figure 5). This clearly shows that there was a negative relationship between normal germinating seedlings and the frequencies of aberrant anaphase cells under different storage conditions, and probit transformation of both variables linearized this relationship in pea landraces as well as in pea cultivars. As the intercept and slope of the regression line for each of the eight storage conditions were not significantly different (P>0.05), a single line was fitted to describe the relationship.

At the start, i.e. 99.99% viability, there were 0.5% aberrations in the seeds but when viability fell to 95% there was a fourfold increase in percentage of aberrations. Thus, even small losses of viability were associated with an increased accumulation of genetic damage.

Further examination showed that in Kelvedon Wonder, at about 82% viability, 12% of aberration accumulated both in the high and low moisture content

seed lots. Similarly, Douce Provence exhibited 9-11% aberrations at 75-70% viability at both moisture contents. In JI 181 at 75% viability the percentage of chromosomal aberration was 16% and in JI 1104 at 40% viability 20% chromosomal aberration were noted again both in high and low moisture content seed lots. Thus, after examining both landraces and cultivars in a range of environments at different temperatures and moisture contents it was found that for a given loss of viability the same frequency of aberrations was observed irrespective of storage conditions.

iii. Types and frequencies of chromosomal aberrations

Tables 1 and 2 illustrate the relationship between storage period and the frequency and type of chromosome damage accumulated. There was general increase in all types of aberrations with decreases in

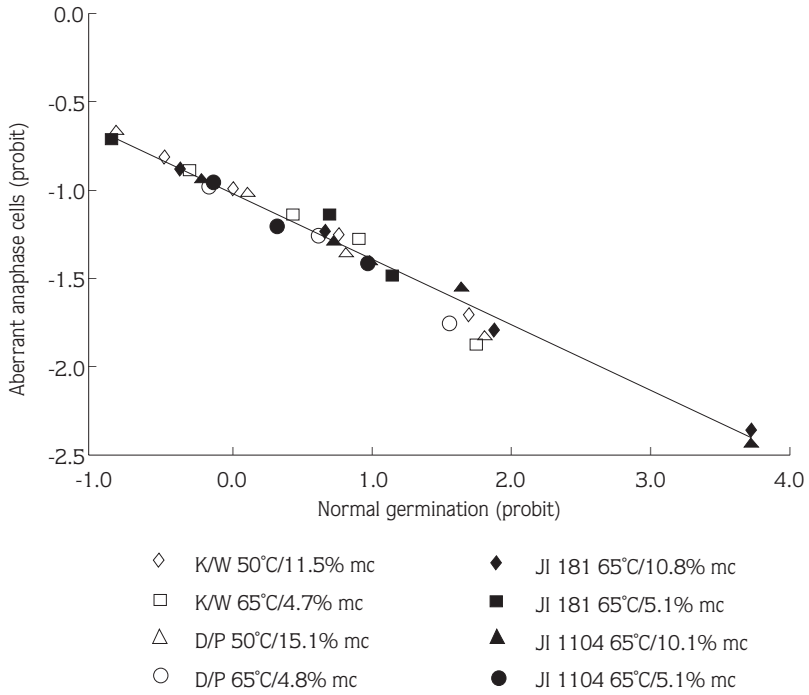

Figure 5. The relationship between percentage normal germination and the total frequency of aberrant anaphase cells in surviving seeds, plotted on probit scale, in pea cultivars and landraces under different storage conditions.

percentage of germination of each cultivar (Table 1) and landrace (Table 2). However, the relative frequency of the various types of aberration with loss of viability varied in the different storage treatments although chromatid-type aberrations were predominant under all the storage conditions in each cultivar and landrace.

It is clear from Table 1 that chromatid-type aberrations were predominant but the proportion of the category "others" was also high in the seeds of cv. "Kelvedon Wonder" stored at 50°C and 11.5% moisture content. A similar trend could be seen in the seeds stored at 65°C and 4.7% moisture content but a high proportion of the category "others" (2.19%) only appeared in the 70 day aged seed lot, which had 38.5% normal germination. The seeds of pea cv. "Douce Procvence" also exhibited similar trends in storage at 50°C and 15.1% moisture content as well as at 65°C and 4.8% moisture content.

In the low moisture content seed lots of JI 181 and JI 1104 (Table 2), the proportion of each aberration category, at any similar viability level, was almost identical to the cultivars with the exception that in the cultivars, storage at high seed moisture contents resulted in higher frequencies of the category "others". In addition, Table 2 shows that in pea landraces, chromosomal aberrations (i.e. chromatid-type aberrations) were present even at 100% seed viability.

Furthermore, mixed-type aberrations only occurred below 80% normal germination in all the seed lots of both cultivars and landraces (Tables 1 and 2).

The data from all the treatments within each seed moisture content of each cultivar and landrace were pooled separately to illustrate the overall frequency of different types of aberration (as percentage of the total number of anaphase cells) accumulated during storage.

The pooled results confirmed that chromatid-type aberrations were predominant at each moisture content level of each cultivar and landrace (i.e. the proportions of chromatid-type aberration to the total frequency of aberrations varied between 73% and 89%). The most common type of chromatid aberration were single fragments (1F), followed by two fragments of unequal size (2F) and single bridges (1B).

Table 1. The relationship between different storage conditions/percentage normal germination and the ratio of cells containing various types of chromosomal aberrations in seeds of pea cultivars.

	Storage Conditions				Chromosomal Aberrations				
Cultivars	Moist cont. (%)	Temp. (°C)	Period (day)	Normal germ. (%)	Chromatid type (%)	Chromosome type (%)	Mixed (%)	Others (%)	Total aberrant cells (%)
Kelvedon Wonder	11.5	50	0	95.5	4.33	-	-	-	4.33
			18	78.0	7.64	0.71	0.36	1.78	10.49
			24	51.0	10.87	1.74	1.30	2.03	15.94
			30	32.0	14.73	2.78	0.66	2.46	20.63
	4.7	65	0	96.0	3.11	-	-	-	3.11
			39	82.0	9.07	0.70	-	0.23	10.00
			50	67.0	11.04	0.82	0.21	0.62	12.69
			70	38.5	14.66	1.09	0.66	2.19	18.60
Douce Provence	15.1	50	0	96.5	2.87	-	-	0.45	3.32
			3.5	84.0	7.67	-	-	0.24	7.91
			4	79.5	7.81	0.36	0.18	0.18	8.53
			6	55.0	12.26	0.24	0.24	2.64	15.38
			7	21.0	20.38	1.42	0.71	2.84	25.36
	4.8	65	0	94.0	3.60	0.15	-	0.15	3.90
			42	73.5	9.17	0.33	-	0.83	10.33
			84	44.0	13.13	1.41	0.31	1.25	16.10

Table 2. The relationship between different storage conditions/percentage normal germination and the ratio of cells containing various types of chromosomal aberrations in seeds of pea landraces.

	Storage Conditions				Chromosomal Aberrations				
Landraces	Moist cont. (%)	Temp. (°C)	Period (day)	Normal germ. (%)	Chromatid type (%)	Chromosome type (%)	Mixed (%)	Others (%)	Total aberrant cells (%)
JI 181	10.8	65	0	100.0	0.88	-	-	-	0.88
			5	97.0	3.59	-	-	-	3.59
			6	75.0	9.63	0.59	0.20	0.39	10.81
			7	36.0	16.28	1.16	0.58	0.78	18.80
	5.1	65	0	100.0	0.99	-	-	-	0.99
			140	87.5	6.77	-	-	-	6.77
			154	76.0	10.89	0.20	0.20	1.39	12.68
			175	20.0	19.96	1.17	0.59	2.15	23.88
JI 1104	10.1	65	0	100.0	0.63	-	-	-	0.63
			6	95.0	5.93	-	-	-	5.93
			8	77.0	7.98	0.80	0.20	0.80	9.78
			10	42.0	14.02	1.48	0.74	1.11	17.35
	5.1	65	0	100.0	0.94	-	-	-	0.94
			140	83.5	7.36	0.29	-	0.20	7.85
			154	63.0	9.57	0.72	0.18	0.90	11.37
			168	45.0	13.99	1.13	0.38	1.32	16.82

Discussion

Inthe present study, data were presented to show that the longevity of cultivated and landrace pea seeds was affected by temperature, seed moisture content and time, i.e. increasing any of these factors decreased viability (Figures 1-3). However, loss of seed viability was slower in the pea landraces compared with the pea cultivars under similar storage conditions (Figure 3). This might be due to the higher initial seed viability (i.e. greater K_i value) or different genotypic characteristics (i.e. tolerance of harsh conditions) of the landraces. Work reported by the International Board for Plant Genetic Resources (IBPGR) (14) on genetic control of storage characters in the pea has suggested that storage life is shorter in white flowered plants than colored ones. The landraces used in this study had colored flowers compared with the white of the cultivars and this phenotypic observation may in part explain the differences in longevity which were observed. However, whatever the reason, it is clear that the storage potential of less selected material, i.e. landraces, is different from that of modern cultivars.

It has been known for some time that there is a negative relationship between loss of seed viability and the accumulation of chromosomal aberrations in diverse species. In peas, this relationship has been confirmed by Abdalla and Roberts (6) and Dourado and Roberts (7). The present results on pea cultivars are consistent with these findings. In addition, this relationship has been shown to apply to the landrace pea seeds as well, i.e. loss of viability is associated with an increase in the accumulation of chromosomal aberrations in landrace seeds as in cultivars. These findings are significant in that this was the first time that investigations had been conducted on non-modern cultivars, in this instance landraces , and the fact that the same relationship held true for landraces as for cultivars lends weight to the principles and practices of seed conservation based on assessments made on cultivars.

Abdalla and Roberts (6), working on barley, broad beans and peas, concluded that the relationship between loss of seed viability and accumulation of aberrant cells during first mitosis was asymptotic, i.e. there was an initial rapid rise in aberrant cells with decrease in seed viability but that as viability decreased below about 50% further increases in the frequency of aberrant cells were hardly detectable. However, in this study, it was shown that in pea cultivars and landraces there was a further considerable increase in the frequency of aberrant cells below 50% viability. This supports Villiers (15), working on lettuce, Murata *et al,* (12, 16), working on barley and Dourado and Roberts (7), working on barley and peas, that the relationship is typically sigmoid.

Both in cultivated and landraces pea seeds, loss of viability and the induction of chromosomal aberrations occurred more rapidly in seeds of high moisture content than low moisture content. In light of previous work on peas it was concluded that even small losses of seed viability were associated with some damage (7). These results confirm this conclusion in the landraces pea seeds as well as in the seeds of pea cultivars. It was shown that small losses of seed viability, e.g. from 100% normal germination (control) to 97% in JI 181 and from 100% (control) to 95% in JI 1104, resulted in some chromosomal damage (i.e. from 0.875% to 3.593% in JI 181 and from 0.626% to 5.925% in JI 1104). In addition, these results also suggest that in pea landraces chromosomal damage is present even at 100% seed viability. These results suggest that "spontaneous" production of chromosomal aberrations is not always the result of ageing and that a background level of aberration can exist in a seed lot.

Previously, Harrison (17) found that lettuce seeds stored at 18°C and 6% moisture content had a much higher frequency of chromosomal aberrations for a given decline in viability compared with seeds stored at 10% moisture content. Recently, Rao *et al,* (3) have shown that in lettuce seeds more chromosomal aberrations were induced at low moisture content (5.5%) compared with a similar viability resulting from ageing at a high moisture content (13.0%). The work presented here on peas contradicts these findings. This study shows that both for cultivars and landraces low moisture content seeds do not exhibit a greater frequency of aberrations than high moisture content seeds of equivalent viability. Rao *et al.* (3) have suggested that lipid peroxidation-mediated-free radical injury might be mainly responsible for the increased chromosome damage at low moisture contents. Pea seeds, however, are high in starch and low in lipids. It is possible, therefore, that lipid peroxidation-mediated-free radical injury in pea seeds is not as effective as in lettuce thus accounting for the differences observed in the accumulation of chromosome damage at low moisture contents in these two species. This is celar evidence of a species different behaviour to the accumulation of genetic damage at low moisture contents.

There is some debate on which type of chromosomal aberration is predominant in aged seeds. Although Abdalla and Roberts (6) have reported the preponderance of chromosome-type aberrations over chromatid-types in

aged seeds of barley, pea and broad bean, more recently, it has been concluded that aberrations in aged seeds were predominantly of the chromatid-type, e.g. in wheat (18), in barley (7, 12, 19) and in peas (7). Recently, Rao *et al.* (20) and Rao and Roberts (21) have shown that in aged lettuce seeds the frequency of chromatid-type aberrations was predominant at high moisture contents whilst the frequency of chromosome-type aberrations was predominant at low moisture contents.

It was confirmed here that chromatid-type aberrations are predominant in the first mitotic divisions of high and low moisture content aged pea seeds. The proportion of chromatid-type aberrations to the total frequency of aberrations was over 84% (with the exception of 72% in the 11.5% m.c. seed lot of cv. Kelvedon Wonder) in each cultivated and landrace pea seed lot. Based on the Exchange Theory of chromosome breakage (7), these findings support the proposal that damage to the DNA occurs as seeds age during storage.

Due to the expense and technology needed to maintain germplasm collections at -18°C the IBPGR is considering storing seeds at ultralow moisture contents, i.e. 1-2% in hermetically sealed containers as many species can then be stored under ambient conditions with extended longevity. These findings in peas that storage at low moisture contents is not associated with an increase in chromosomal aberrations as seen in lettuce lends weight to the proposal and stresses the need to examine whether this is indeed a universal phenomena or one just peculiar to oily seeds such as lettuce.

As mentioned earlier, the discovery that landraces exhibit similar storage behaviour to cultivars is further reassurance that the guidelines for genetic resources conservation which have been based only on work on cultivars are sound. Knowing that certain landraces exhibit greater longevity, pea germplasm should be screened for this character and an attempt should be made to isolate this trait.

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